slip transferred to a tube of litmus dextrose broth. Growth occurred within 24 hours.

So far as the possibility of interplanetary bacterial life is concerned, then, it is evident that bacteria in the fully dried state, if free in the interplanetary vacuum, would be killed by the solar light and ultra-violet rays.

And, as Sir James Dewar's experiments have demonstrated that the ultra-violet rays will kill undried bacteria whilst in the frozen condition, at the temperature of liquid air (−190° C.), there is little to support the hypothesis that the living protoplasm on the earth originally immigrated from interplanetary space in a free or unincluded condition—that free, particulate life has entered the earth's atmosphere as a result of light propulsion, from extra-mundane space.

An Improved Method for Opsonic Index Estimations, involving the Separation of Red and White Human Blood Corpuscles.

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Opsonin is the name given by Sir A. E. Wright to the substance in human blood which mainly influences phagocytosis. In order to measure the opsonin, equal volumes of normal serum, of a bacterial emulsion, and of leucocytes are incubated to promote phagocytosis, and the number of bacteria visible in a random sample are counted in a stained film preparation. By repeating the experiment with a pathological serum, a ratio is obtained which is known as the opsonic index. The method, however, has been found inaccurate, and consequently fallen almost into disuse. Drs. Greenwood and White pointed out the error of random sampling and, after elaborate mathematical analysis, adjudged the "liability to error" on a single count to occasionally exceed 20 per cent. from the mean or true figure. Clearly, when the counts from two films are contrasted, the liability to error may be doubled.

This liability to error is due to the variation in content of different leucocytes in the same film ranging from 0 to 25 bacteria, and the resulting total in 50 leucocytes will vary according to the field in which the operator happens to make his count, *i.e.*, one rich in high or low contents.

In my work this error has been studied and largely reduced. The first idea was that the number of leucocytes in the mixture might vary in the successive sets of materials. To test this point I repeatedly estimated the opsonin of normal serum to staphylococci, and also counted the leucocytes per cubic millimetre in each opsonic mixture. The results are shown in Table A.

Table A.—Opsonic Index. (Old Method.)

Repeated estimation of the opsonin to *Staphylococcus aureus* in normal serum and simultaneous enumeration of the leucocytes per cubic millimetre in each opsonic mixture.

Series.	Leucocytes per cu. mm.	Opsonic count 50 leucocytes.	Error *above* average.	Error *below* average.	Mean value.	Blanks per 50 leucocytes.	Worst error *above* average.	Worst error *below* average.
			per cent.	per cent.			per cent.	per cent.
1	15 × 200	279	—	4	292	9	—	—
(2 6 / 1)	9 ,,	337	15	—		9	15	—
	14 ,,	285	—	2		7	—	—
	11 ,,	268	—	8		6	—	8
2	18 ,,	190	11	—	170	10	—	—
(1 6 / 1)	16 ,,	258	52	—		3	52	—
	19 ,,	118	—	30		20	—	—
	10 ,,	114	—	33		20	—	33
3	14 ,,	185	8	—	170	16	—	—
	9 ,,	155	—	8		14	—	—
(2 3 / 1)	6 ,,	213	25	—		13	25	—
	16 ,,	137	—	19		21	—	19
	36 ,,	180	5	—		13	—	—
	12 ,,	155	—	8		14	—	—
4	12 ,,	151	—	20	190	11	—	20
(1 5 / 1)	7 ,,	191	—	—		7	—	—
	25 ,,	191	—	—		8	—	—
	10 ,,	224	17	—		4	17	—
	Total.....		133	132				

Average error above or below the mean value = 265/18 = 14·7 per cent.
Worst error *above*..... = 52 ,,
Worst error *below*..... = 33 ,,
The encircled numbers indicate the highest leucocyte content in each series and the lower figure = the number of times it occurred.

All the materials were identical in each series, and the opsonic estimations by the same operator, and the observations made at about half-hour intervals.

The following points are apparent from these experiments :—

(1) The leucocytes did vary in the series used for the opsonic estimations.

(2) No definite relationship was apparent to a high or low count from this leucocyte variation.

(3) The opsonic count often showed large variations from the mean value, *i.e.* a large experimental error.

(4) There appeared to be the all-important constancy of the opsonic figure when the numbers of blanks met during a 50 count was the same or nearly so.

This last feature suggested that this large content variation might be due to uneven access between leucocytes and bacteria. However, the outstanding fault of the process is this large variation in the leucocyte content, and its occurrence could only be due to differences of (1) *appetite* or (2) *opportunity*, or a combination of these factors.

(1) *Appetite.*—If the leucocytes have equally as good chances to pick up bacteria in the opsonic mixture, and yet show this variation, it must be presumed physiological, and there is no remedy.

(2) *Opportunity.*—It may be that all the leucocytes have similar appetites, but get very different opportunities to pick up bacteria, owing to their uneven distribution in the mixture.

A scrutiny of the materials used in the old method showed two important defects.

The Presence of Red Corpuscles.—Although white corpuscles only are concerned in the process, both red and white blood corpuscles are used ; but, since a bacterial suspension not exceeding 500M per cubic centimetre is used, and washed blood corpuscles contain 5000M red and 10M white corpuscles per cubic centimetre, it is evident that for every leucocyte there are 50 bacteria provided, but surrounding this all-important leucocyte are 500 obstructing and useless red corpuscles. I therefore decided to abolish the red corpuscles, and this involved the *separation of red and white human blood corpuscles.*

The methods which were tried unsuccessfully included—Hæmolysis of the red corpuscles ; agglutination by ferric chloride and filtration of the red groups ; filtration of decalcified blood ; sedimentation of decalcified blood after artificially raising the specific gravity.

However, Dr. Ponder's work on leucocytes furnished the nucleus of a successful method. He found that when blood is enclosed in a cell between two glass plates and incubated, there occurs a swarming of polynuclear leucocytes to the glass surfaces, to which they adhere firmly and appear remarkably distorted.

My first problem was to get these leucocytes off the glass. This was found to be effected by citrate saline solution 1·5 : 0·8 per cent., by disodium hydrogen phosphate, and by hypertonic salt solution, and also by serum. After obtaining a large number in a test-tube in the citrate solution (to make films), I found they were highly unstable osmotically and, when transferred to normal saline, the majority burst.

After experimenting with over 200 Ponder plates, I realised that the leucocytes could only be obtained in bulk if favourable chemical conditions were ascertained (since incubation aggravated the bursting). The following method succeeded in supplying a majority of polynuclear leucocytes (the lymphocytes do not appear on the plates) in good condition, which could be incubated for 15 minutes, as in the opsonic index process. The detailed method is as follows :—

Blood is shed into a rubber ring (cell) sandwiched between two glass plates. This cell is incubated for 20 minutes at 37·4° C., removed from incubation, the cell is opened, the clot and ring removed, the plates washed with 1·25-per-cent. saline, to free them from red corpuscles and serum. After wiping the ring margin clear of more red corpuscles and dried serum a few drops of cold NaCl (1·25 per cent.) are poured on the leucocyte-laden area of each plate. These are replaced on the metal shelf of the incubator for 15 minutes; when the plates are inspected the previously distorted and stretched out polynuclears will be seen under a low objective to have become almost spherical and loose from the plate. By means of a long glass rod the fluid and floating leucocytes are swept into a small tube and concentrated by the centrifuge at moderate speed. After syphoning off the supernatant fluid a very large number of human polynuclear leucocytes were obtained, which stand incubation with equal volumes of serum and the bacterial emulsion, the latter being made with 1·25-per-cent. NaCl instead of normal saline.

But before proceeding to test the opsonic process for the anticipated increased accuracy the second defect of opportunity in the old method had to be attended to. In the old method the opsonic mixture (serum, bacteria, and washed blood corpuscles) was incubated for 15 minutes, but even at the end of 10 minutes the bulk of the corpuscles had settled to the bottom of the glass pipette, the supernatant fluid being clear. Since equality of opportunity cannot exist when this is permitted this defect was remedied by keeping the mixture in slow rotary motion during incubation by means of the opsonic mill (fig. 1), and by this device no sedimentation occurs, but there is some degree of active mixing.

A mechanism to prevent sedimentation of the opsonic mixture was devised by Rosenow, 1906, and by Glynn and Cox, 1912.

The latter used a mechanism which rotated the pipette on its long axis in a horizontal plane, and their experiments showed no reduction of the error by its use when tested.

Only a small benefit is to be expected from such an improvement, but it is probably inappreciable when the entire experimental error may be large, as in the old method.

Moreover, their method of rotation is not ideal, for though sedimentation

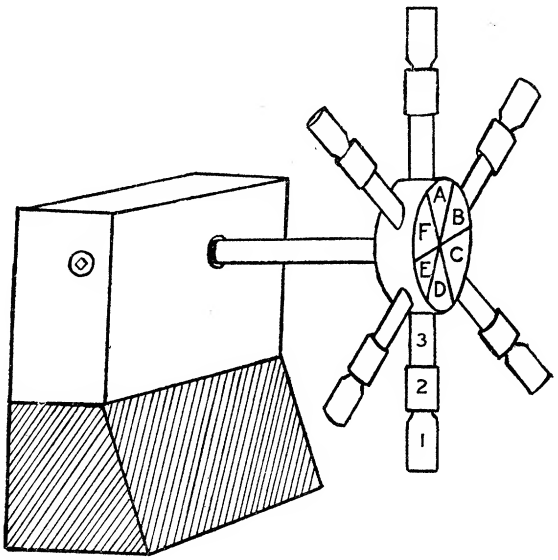

FIG. 1.—The Opsonic Mill.

This clockwork-driven instrument standing in the incubator rotates the opsonic mixture and prevents sedimentation of the corpuscles. Speed of rotation = 1 min. 45 secs. per revolution. (1) The shortened opsonic pipette. (2) Indiarubber collar. (3) Copper tube lined with fine copper wires. The brake is not illustrated.

is prevented, there is no active mixing (from end to end of the pipette) induced by such a roller movement. In the device illustrated in fig. 1, not only does no settling occur, but experiments showed that the corpuscles pass up and down in the opsonic fluid during the changing positions of the pipette in the slowly moving wheel.

After a few trial experiments with the new materials I proceeded to test the opsonic index of the same serum repeatedly, as in the experiments recorded (Table A), to ascertain whether the more even access and mixture of bacteria and leucocytes now improved the experimental error.

The results are shown in Table B.

Table B.—Opsonic Index. (New Method.)

Repeated estimation of the opsonin to *Staphylococcus aureus* in normal serum, using leucocytes only and the opsonic "mill."

The series number indicates a set of four or six observations made at a sitting with the same set of materials.

Series.	Opsonic count, 50 leucocytes.	Error *below* average.	Error *above* average.	Mean value.	Blanks, per 50 leucocytes.	Worst error *above* average.	Worst error *below* average.
		per cent.	per cent.			per cent.	per cent.
1. Very thin bacterial emulsion	61	16	—	72	27	—	16
	72	—	—		17	—	—
	76	—	5		21	—	—
(7/1)	79	—	9		19	—	—
	67	6	—		20	—	—
	84	—	15		14	15	—
2. Thin bacterial emulsion	117	—	13	103	13	13	—
	103	—	—		13	—	—
	114	—	11		12	—	—
(12/2)	87	15	—		11	—	15
	91	11	—		10	—	—
	108	—	4		18	—	—
3. Medium bacterial emulsion	130	6	—	139	6	—	6
	147	—	5		9	5	—
	135	2	—		11	—	—
(10/1)	144	—	3		8	—	—
4. Thick bacterial emulsion	160	10	—	178	4	—	10
	195	—	9		3	—	—
	197	—	10		6	—	—
(14/1)	163	8	—		7	10	—
Total		74	84				

Average error above or below the mean value = 158/20 = 7·9 per cent.
Worst error *above* the mean value..... = 15 „
Worst error *below* the mean value..... = 16 „

The results recorded in this table (B) show a marked reduction of the average and maximum error from the mean value, and this had occurred in spite of a fairly wide variation in the strength of the bacterial emulsion used. Believing that still higher accuracy might be obtained I undertook a further series of tests, matching the emulsion used in Experiment 3, Table B (which had been fixed by heat), as a standard. I also decided to count 100 leucocytes, 50 from each of the two films made from the mixture.

The results are shown in Table C, and show a still higher level of accuracy than those of Table B, though visual matching of the emulsions (to produce an average phagocytosis of three bacteria per leucocyte) was not very successful, though it can be assured in future by a preliminary count.

Table C.—Opsonic Index. (New Method.)

Repeated estimation of opsonin in normal serum to the *Staphylococcus aureus*, using leucocytes only, and the opsonic mill, and counting 100 leucocyte contents.

Series.	50 leucocytes, Film I.	50 leucocytes, Film II.	Opsonic count.	Mean value.	Error from mean value.		Blanks, per 100 leucocytes.	Maximum deviation from mean value.	
					+	−		+	−
					Per cent.	Per cent.		Per cent.	Per cent.
1	144	105	249	256	—	2	18	—	2
(10/2)	127	144	271		6	—	18	6	—
	107	146	253		—	1	16	—	—
	131	119	250		—	2	20	—	—
2	142	178	320	324	—	1	10	—	—
(14/1)	170	159	329		1	—	15	—	—
	150	160	310		—	3	16	—	3
	176	162	338		4	—	8	4	—
3	127	120	247	252	—	2	49	—	—
(14/1)	128	103	231		—	8	40	—	8
	144	113	257		2	—	32	—	—
	144	132	276		9	—	40	9	—
4	99	112	211	230	—	8	25	—	8
(9/4)	114	144	258		12	—	19	12	—
	121	109	230		—	—	23	—	—
	96	125	221		—	3	25	—	—
			Total		34	30			

Average error above or below the mean value = 64/16 = 4 per cent.
Worst error *above* ..... = 12 „
Worst error *below* ..... = 8 „

There was defective emulsification of the staphylococci in Series 3, evident in the "clumpy" films, but fortunately the entire results of the table can afford the handicap.

All the films of Tables B and C have been preserved.

Summary.

The improvements described have produced:—

(1) A striking reduction in the liability to error of opsonic estimations when repeatedly tested.

(2) The results recorded by the new method (Table C) showed a liability to error of about one quarter the magnitude of those recorded in Table A (old method), the experimental conditions being almost comparable.

(3) The enhanced accuracy is associated with a much reduced range of microbic content of the leucocytes (0—14).

(4) The improved results are attributable to the more even distribution of bacteria amongst the leucocytes (by the removal of the red corpuscles) and by its maintenance during incubation in the opsonic mill.

(5) No observations were made of any variations in opsonin in health or pathological states.

I am indebted to Dr. Cavendish Fletcher for his valuable assistance with this work, which unfortunately he had to abandon.

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